

EVALUATING THE POTENTIALITY OF LEAVES OF *MANILKARA ZAPOTA* (L) *P.ROYAN AND MIMUSOPS ELENGI* L. IN THE SYNTHESIS OF SILVER NANOPARTICLES

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ABSTRACT

The present study is aimed to evaluate the potentiality of leaves of two different higher plants, *Manilkara zapota* and *Mimusops elengi* in the synthesis of silver nanoparticles. The exposure of these leaf broths separately into aqueous silver nitrate solution changed their color from pale yellow to brown and finally became dark brown colour. It indicates the reduction of silver nitrate into silver nanoparticles. The synthesized silver nanoparticles were characterized by the UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray (EDX) spectroscopy, Transmission Electron Microscopy (TEM) and Atomic Absorption Spectroscopy analysis. On evaluation, it is revealed that the leaf broth of reaction medium of *M. zapota* produces more silver nanoparticles with much smaller in size than the leaf broth of reaction medium of *M. elengi*. This is due to more availability of reducing and capping agents in the leaf broth of *M. zapota* than that in the leaf broth of *M. elengi*.

KEYWORDS: *Manilkara zapota*, *Mimusops elengi*, Biomaterial, Reaction Medium, Silver Nanoparticles

INTRODUCTION

Nanoparticles generally considered as particles with a size of up to 100 nm exhibit completely new or improved properties that are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles [1]. The protocol for the green synthesis of silver nanoparticles is utilizing relatively non-toxic solvents such as water, biological extracts and biological systems [2]. Many researchers used various biological systems for the green synthesis of silver nanoparticles such as sea weed [3], fungi [4, 5], bacteria [6], actinomycetes [7] and higher plants [8- 10]. The higher plants have been exploited for the synthesis of silver nanoparticles due to their potential reducing capacity, more availability of reducing agents, possibility of faster rate of synthesis and also lesser steps in downstream processing, thereby making the process cost effective[11 - 15]. The use of plants for the synthesis of silver nanoparticles is not only cost effective but also generates good quality and more quantity of nanoparticles within few hours and can be used for the various medical applications [16, 17]. Hence the present study is aimed to evaluate the potentiality of leaves of *M. zapota* and *M. elengi* in the reduction of silver nitrate into silver nanoparticles.

MATERIALS AND METHODS

All the reagents used in the present study were obtained from Himedia Laboratories Pvt Ltd (Mumbai, India). The fresh and healthy leaves of *M. zapota* (Figure 1a) and *M. elengi* (Family: *Sapotaceae*) (Figure 1b) were collected from

the Botanical Garden of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India. The collected leaf samples were thoroughly washed with tap water followed by distilled water to remove the surface contaminants and dried for 48 hours under shade. The dried leaves were taken separately and ground to make fine powder using mixer and the sieved using 20 mesh sieve to get uniform size range. For the preparation of *M. zapota* leaf broth, the sieved leaf powder of *M. zapota* (10g) was added to 100ml of distilled water and boiled at 70°C for ten minutes [14, 18]. The extract was filtered and the leaf broth was obtained. The freshly prepared leaf broth (10 ml) was re suspended in 190ml of 1mM aqueous solution of silver nitrate and this mixture is used as reaction medium. The reaction medium was kept in an Incubator cum shaker (Orbitek-Model) with 250 rpm at 27°C for 24 hours. Similarly, the same procedure was repeated for the preparation of *M. elengi* leaf reaction medium. From these reaction media a small aliquot of the samples was collected separately to characterize the silver nanoparticles that were synthesized during the above reaction.



Figure 1a: Leaves of *M. zapota*

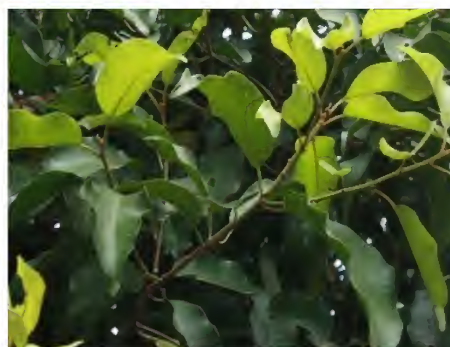


Figure 1b: Leaves of *M. elengi*

CHARACTERIZATION OF SILVER NANOPARTICLES

The characterization of synthesized silver nano particles was performed through the following analyses: UV-Vis spectroscopy (Labomed Model UV- D3200), Spectrofluorimetric analysis (Elico), FT-IR analysis (Shimadzu), XRD analysis (Shimadzu, XRD 6000), SEM (Hitachi S-4500) coupled with EDX analysis and TEM analysis (Philips-Techno 10 instrument operated at an acceleration voltage of 200KV with resolution of 0.3nm). The quantity of silver nanoparticles synthesized by leaf tissue was estimated using Atomic Absorption Spectroscopy (AAS) in accordance with the following equation [19].

$$q = [(C_0 - C) / X]$$

Where: q (mg of metal nanoparticles synthesized by one gram of leaf tissue) is the metal specific uptake, C_0 is the initial metal concentration (mg l^{-1}), C is the residual metal concentration (mg l^{-1}) and X is the biomass concentration of the leaf tissue (g).

RESULTS AND DISCUSSIONS

UV-Visible Spectrum of Silver Nanoparticles

The preliminary technique in the characterization of silver nanoparticles is UV-Vis spectroscopic analysis. The fresh leaf broth of *M. zapota* was pale yellow in colour. When exposed to aqueous silver nitrate, the leaf broth of *M. zapota* turned its colour, pale yellow to brown within ten minutes and became dark brown in colour within twenty four hours of incubation (Figure 2a inset). Simultaneously the pale yellow colored leaf broth of *M. elengi* when exposed to aqueous silver nitrate it turned to brown within thirty minutes and dark brown after six hours of incubation

(Figure 2b inset). The time taken for the complete reduction of silver nitrate into silver nanoparticles varied from plant to plant which used as reducing agent. For example, leaves of *Ipomoea pescaprae* took twenty five hours [20], four hours by flowers of *Millingtonia hortensis* [21], ninety six hours by the entire plant of *Rumex hymenosepalus* [22], forty eight hours by the entire plant of *Cassia italica* [23] and seventy two hours by fungus *Rhizopus stolonifer* [24]. This color change is due to the excitation of Surface Plasmon Resonance (SPR) vibrations of synthesized silver nanoparticles [25].

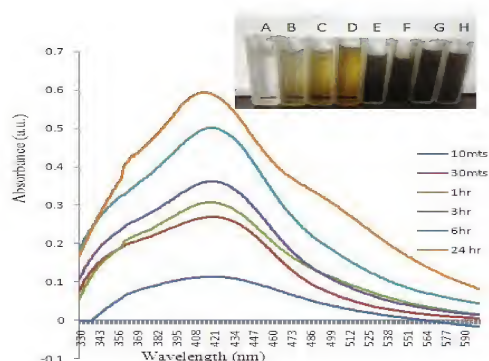


Figure 2a: UV –Visible Absorption Spectra of Silver Nanoparticles Synthesized by Leaf Broth of *M. zapota*. The Inset Shows the Colour Change of the Reaction Medium (Left to Right) A-Control (Aqueous Silver Nitrate); B- Leaf of *M. zapota*, D, E, F, G and H are the Reaction Media at Different Time Intervals of Reaction Such as 10 Minutes, 30 Minutes, One Hour, Three Hours, 6 Hours and 24 Hours Respectively

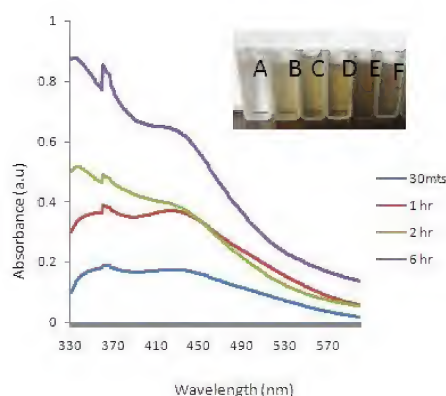


Figure 2b: UV –Visible Absorption Spectra of Silver Nanoparticles Synthesized by Leaf Broth of *M. elengi* L. The Inset Shows the Colour Change of the Reaction Medium (Left to Right) A-Control (Aqueous Silver Nitrate); B- Leaf Broth of *M. elengi* L. C, D, E, F and G are the Reaction Media at Different Time Intervals of Reaction Such as 30 Minutes, One Hour, Two Hours, 4 Hours and 6 Hours Respectively

The UV- VIS spectrum recorded from 300 to 600 nm for the silver nanoparticles synthesized using the leaf broth of *M. zapota*, shows a λ max at 420 nm and the absorbance raised up to 0.6a.u. at twenty four hours incubation (Figure 2a); In the reaction medium with leaf broth of *M. elengi*, the SPR band of λ max observed at 425 nm and the absorbance raised up to 0.7a.u. at six hours incubation (Figure 2 b). The λ max of SPR observed for silver nanoparticles may vary with an organism by which they are synthesized. The λ max of silver nanoparticles synthesized by the seaweed *Kappaphycus alvarezii* [3] and *Cassia angustifolia* leaf extract [26] and *Paederia foetida* leaf extract [13] and *Odina wodier* leaf extract[15] respectively was 450 nm. Interestingly, *M. zapota* took twenty fours for the complete reduction of silver nitrate into silver nanoparticles whereas in *M. elengi* took six hours for the complete reduction.

SPECTROFLUORIMETRIC ANALYSIS

Figures 3a and 3b show the respective emission and excitation spectra of leaf broth reaction media of *M. zapota* and *M. elengi* respectively. Silver nanoparticles synthesized using leaf broth of *M. zapota* show that the excitation peak is found at 420 nm, while the emission peak is observed at 450 nm (Figure 3a). The silver nanoparticles synthesized using the leaf broth of *M. elengi* show the excitation peak at 425 nm, while emission peak at 440 nm (Figure 3b). The excitation peaks at 420 nm and 425 nm by the silver nanoparticles synthesized by *M. zapota* and *M. elengi* respectively, are coincided well with the absorption maxima (λ max) recorded with UV-Vis spectra. In the spectrofluorimetric analysis of silver nanoparticles synthesized using *Nicotiana tabacum*, the excitation peak at 414nm and emission peak at 576nm were noticed. [27] noticed. The quantum yield obtained in the reaction medium with *M. zapota* leaf broth is 0.982 while that in the reaction medium with *M. elengi* leaf broth is 1.014.

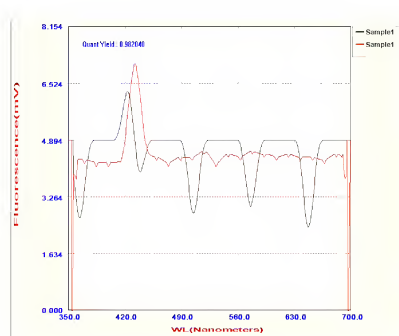


Figure 3a: Spectrofluorimetric Analysis of Silver Nanoparticles Synthesized by Leaf Broth of *M. zapota*

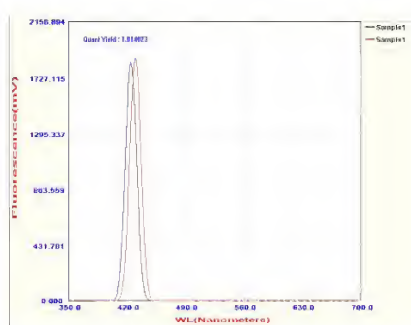


Figure 3b: Spectrofluorimetric Analysis of Silver Nanoparticles Synthesized by Leaf Broth of *M. elengi*

FTIR SPECTROSCOPIC ANALYSIS

Figure 4a shows the FTIR spectrum of silver nanoparticles synthesized using the leaf broth of *M. zapota*. The FTIR bands observed at 605.61, 653.82, 752.19, 806.19, 1112.85, 1193.85, 1336.58, 1398.30, 1452.30, 1593.04, 1668.31, 2268.13, 2682.80, 2885.31, 2972.10, 3193.90 and 3313.48 cm^{-1} respectively. Figure 4b shows the FTIR spectrum of silver nanoparticles synthesized using the leaf broth of *M. elengi*. The prominent peaks observed at 603.68, 752.19, 810.05, 112.85, 1195.78, 1336.58, 1398.30, 1454.23, 1633.59, 1670.24, 2113.64, 2268.13, 2883.38, 2974.03, 3193.90 and 3313.48 cm^{-1} respectively. The peaks at 653, 1595 and 2682 cm^{-1} are found in the reaction medium of *M. zapota* may be associated with the stretching vibrations of $-\text{C}-\text{C}-\text{C}$ in aromatic ring [28] which are not found in *M. elengi*. The two different peaks at 2113 and 1633 cm^{-1} found in the reaction medium of *M. elengi* represent in carbonyl groups ($\text{C}=\text{O}$) from polyphenols such as catechin gallate, epicatechin gallate and the aflavin [29]. These biomaterials found in both the reaction media may act as reducing agents and capping agents for the synthesis and stabilization of silver nanoparticles.

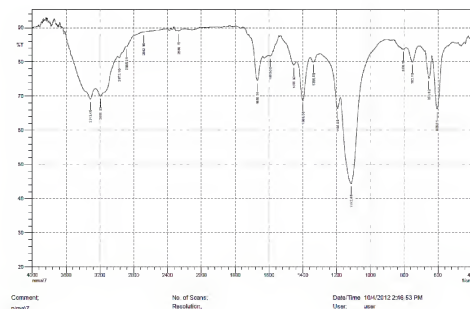


Figure 4a: FTIR Spectrum of Synthesized Silver Nanoparticles Using Leaf Aqueous Broth with Silver Nitrate of *M. zapota*

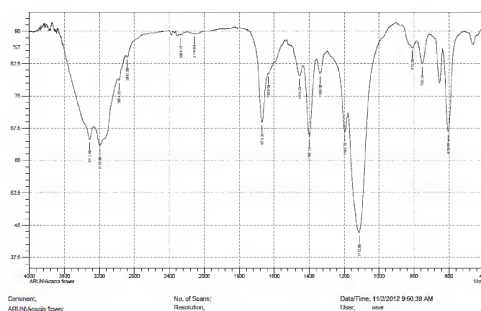


Figure 4b: FTIR Spectrum of Synthesized Silver Nanoparticles Using Leaf Aqueous Broth with Silver Nitrate of *M. elengi*

XRD ANALYSIS

Figure 5a shows the X-ray diffraction patterns of silver nanoparticles synthesized using the leaf broth of *M. zapota* show five intense peaks of 2θ values 27.79° , 32.20° , 38.10° , 44.3° and 64.4° . The 2θ value at 27.79° marked within (220), 32.20° marked within (111), 38.10° marked within (200) 44.3° marked within (220) and 64.4° marked within (311) corresponds to face centered cubic (fcc) structure of silver [30, 31]. The XRD spectrum of silver nanoparticles synthesized by *M. elengi* (Figure 5b) show six intense peaks of 2θ values corresponding to 27.74° (220), 32.15° (111), 38.06° (220), 44.24° (220), 46.14 (220) and 64.14° (311) represent nanocrystals [2]. The crystalline size of silver nanoparticles was calculated from the width of the XRD peaks, using Debye-Scherrer formula $D=0.94\lambda/\beta \cos \theta$ where D is the average crystallite domain size perpendicular to the reflecting planes. λ is the x-ray wavelength, β is the full width at half maximum and θ is the diffraction angle [32]. The average size of silver nanoparticles synthesized using leaf broth of *M. zapota* is 10 nm while that of *M. elengi* is 15 nm.

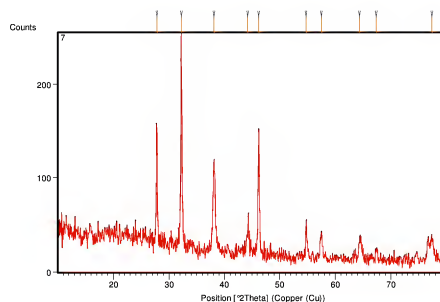


Figure 5a: XRD Pattern of Silver Nanoparticles Formed after Reaction of Leaf Broth of *M. zapota*

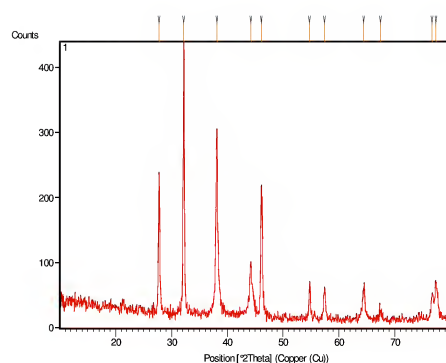


Figure 5b: XRD Pattern of Silver Nanoparticles Formed after Reaction of Leaf Broth of *M. elengi*

SEM AND EDX ANALYSIS

The SEM image of silver nanoparticles synthesized using *M. zapota* (Figure 6a) reveals that the obtained particles are cubical and relatively spherical in shape with a diameter range of 20-40nm. The silver nanoparticles synthesized using leaf broth of *M. elengi* show more or less spherical shaped silver nanoparticles with a diameter range of 30 -50nm (Figure 6b). The synthesis of silver nanoparticles which are with different dimensions and shapes have been achieved using different plants. For example, the relatively uniform shaped silver nanoparticles with a diameter range from 20-40nm were obtained using the leaf extract of mulberry [33]. The spherical shaped silver nanoparticles were synthesized by *Euphorbia hirta* [34] and *Amaranthus polygonoides* [28] with the diameter 40-50nm; *Cassia auriculata* with the diameter 20nm [35]; *Cajanus cajan* [36] with the diameter 40 nm.

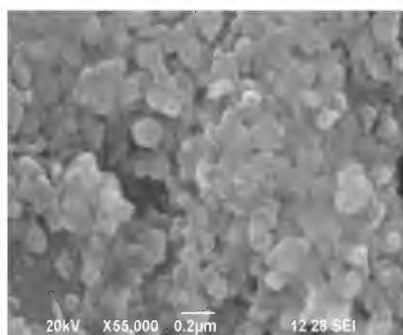


Figure 6a: SEM Images of Silver Nanoparticles Synthesized from the *M. zapota*

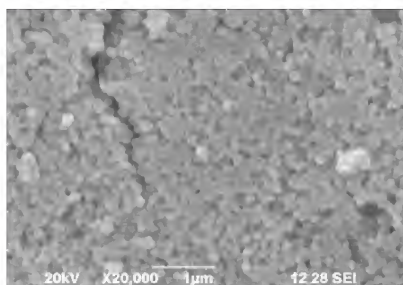


Figure 6b: SEM Images of Silver Nanoparticles Synthesized from the *M. elengi*

Further EDX spectrum of silver nanoparticles synthesized using leaf broth of *M. zapota* (Figure 7a) shows as strong elemental signal of silver nanoparticles (68%) along with the weak O (14%) and Cl (17%) bonds. The EDX spectrum of silver nanoparticles synthesized using leaf broth of *M. elengi* (Figure 7b) shows the presence of an elemental

silver signal Ag (69%) along with weak Cl (22%) S (0.48%), Na (2.18%) and O (4.94%) bonds. The weak signals may possibly due to elements from enzymes or proteins present within the leaf broth [37]. In the present study it is believed that the enzymes or proteins of the leaf broth of *M. zapota* and *M. elengi* may act as the reducing and capping agent for the synthesis of silver nanoparticles. The EDX spectrum showed that the elemental silver (68%) produced by *M. zapota*, which is almost similar to that produced by *M. elengi* (69%).

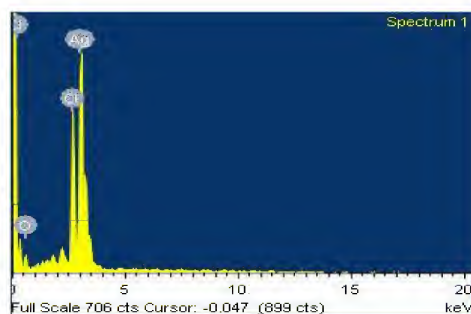


Figure 7a: EDX Images of Silver Nanoparticles Synthesized from the *M. zapota*

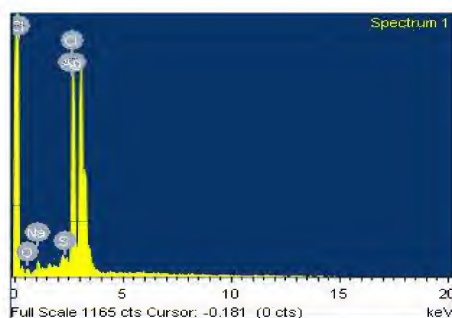


Figure 7b: EDX Images of Silver Nanoparticles Synthesized from the *M. elengi*

TEM ANALYSIS

Figure 8a shows the TEM image of silver nanoparticles using synthesized leaf broth of *M. zapota*. The nanoparticles obtained are polydispersed with size and shape varying between 05 and 50nm with the mean 10.0 ± 0.58 nm (Figure 8b) The silver nanoparticles synthesized using leaf broth of *M. elengi*, show polydispersed and spherical in shape (Figure 9a). The particle size is varying between 05 to 30nm with the mean 15.0 ± 0.753 nm is shown in (Figure 9b). Among the plants that were used in the synthesis of silver nanoparticles, *Cassia angustifolia* [28], *Memecylon umbellatum* [38] and *Pulicaria glutinosa* [39] had produced spherical silver nanoparticles with the average size 10- 15 nm [28], 15-20 nm [38] and 40-60 nm [39] respectively.

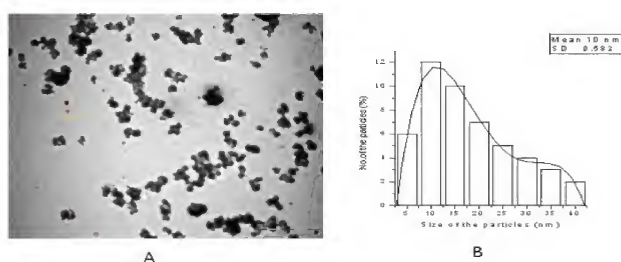


Figure 8a: TEM Images of Silver Nanoparticles Synthesized from the *M. zapota* Size Distribution of Silver Nanoparticles Synthesized from the *M. zapota* Measured by TEM Analysis

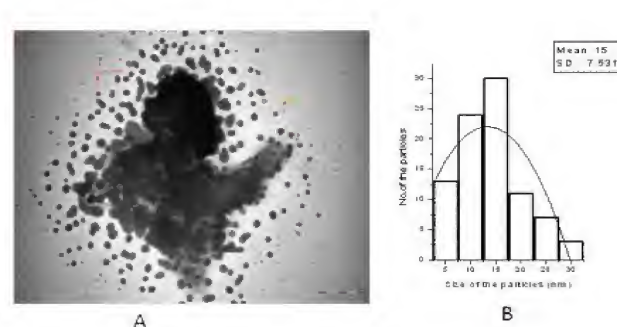


Figure 8b: TEM Images of Silver Nanoparticles Synthesized from the *Mimusops elengi* L. B) Size Distribution of Silver Nanoparticles Synthesized from the *Mimusops elengi* L. Measured by TEM Analysis

ATOMIC ABSORPTION SPECTROSCOPY

The amount of silver nanoparticles synthesized using *M. zapota* and *M. elengi*, was estimated through AAS. It is found that one gram dry weight of leaves of *M. zapota* can synthesize 1.31mg while one gram dry weight of leaves of *M. elengi*, can synthesize 1.09 mg of silver nanoparticles. The synthesis of silver nanoparticles using *M. zapota* is the better source when compared to *M. elengi*.

CONCLUSIONS

The present study evaluates the potentiality of leaves of *M. zapota* and *M. elengi* in the synthesis of silver nanoparticles. *M. zapota* took longer time than *M. elengi* for the complete reduction of silver nitrate into silver nanoparticles. However, the leaves of *M. zapota* could produce smaller nanoparticles (with the size of 10.0 ± 0.58 nm) than that of *M. elengi* (with the size of 15.0 ± 0.753 nm). Further the leaves of *M. zapota* could produce more amount of nanoparticles (1.31mg per g.dw⁻¹ of leaves) than that of *M. elengi* (1.09mg per g.dw⁻¹ of leaves). On evaluation of the potentiality of those two leaves, it is found that the leaves of *M. zapota* have great potentiality than that of *M. elengi* in the synthesis of silver nanoparticles.

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